A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo

P. Bonfante; M.A. Selosse. A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo. NEW PHYTOLOGIST. 186(2) pp: 267-270.
DOI: 10.1111/j.1469-8137.2010.03196.x

A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo

Authors

Paola Bonfante, Marc-André Selosse

First published: 25 March 2010

DOI: 10.1111/j.1469-8137.2010.03196.x

Arbuscular mycorrhizal (AM) fungi (AMF) have entered the mainstream of biology only in recent times, as a result of developments in DNA technologies and genomics, which are providing new tools to identify symbiont diversity, communication and development, and to reveal the contribution of each partner to the functioning of the symbiosis (Parniske, 2008; Bucher *et al.*, 2009). In this issue of *New Phytologist*, Bin Wang and colleagues (pp. 514–525) indirectly demonstrated the antiquity of the plant–AM fungal association. They elegantly used molecular tools, in an evo-devo manner, to show that this widespread association is probably homologous in all lineages of land plants (Embryophyta).

'Ironically, the mycorrhizal association is more ancient than the roots defining it (-rhiza), which arose within vascular plants only.'

Arbuscular mycorrhizas and other symbioses in land colonization

After surveying past and recent literature in this field, even an inattentive reader can quickly identify a few re-occurring assumptions on AM associations that are widely shared by the scientific community. One is that AMF improve plant nutrient uptake through the fine exploration of the rhizosphere and nearby soil by hyphae, whose network can extract nutrients that are otherwise not available to the plant. This has been soundly supported by many investigations, which have convincingly demonstrated the nutrient transfer and the underlying mechanisms (Bucher *et al.*, 2009). Equally popular is the statement that AMF, that is the Glomeromycota, have co-evolved with plants for at least the last 400 million yr, and that this association allowed the conquest of dry terrestrial ecosystems by Embryophyta (Selosse & Le Tacon, 1998; Wang & Qiu, 2006). Recent re-analyses of early enigmatic terrestrial fossils suggest that the first macroscopic land colonizers were fungal–algal associations, such as *Prototaxites* (Selosse, 2002) or *Spongiophyton* (Taylor *et al.*, 2004). Indeed, adding an alga (which is able to exploit light and CO_2 from the environment) to a fungus (able to exploit the soil substratum) makes a perfect adaptation to living at the soil–atmosphere interface, the landmark of land plants. Evidence supporting the antiquity of plant–AMF associations have so far been limited to molecular clock analyses using DNA sequences (Simon *et al.*, 1993), and to fossils analyzed in the original paper by Kidston & Lang (1921) and in the overlooked paper by Boullard & Lemoigne (1971), both based on the 400-million-yr-old Rhynie Chert deposits. However, the Rhynie Chert deposits are more recent than the earliest land plants (Wellman *et al.*, 2003), and none of the previous indirect evidence provides insights into the functioning of the association.

Interestingly, AMF colonize extant basal plant lineages, that is, some of the paraphyletic 'bryophytes' (liverworts = hepatics and hornworts; Russell & Bulman, 2005; Ligrone *et al.*, 2007), as well as the Lycophyta (Winther & Friedman, 2008) and ferns (Boullard, 1979). This stimulated comments on the association of plant ancestors with AMF (Kottke & Nebel, 2005; Selosse, 2005; Leake *et al.*, 2008) and on the capacity of AMF to colonize plant tissues irrespectively of the gametophytic or sporophytic status (Bonfante & Genre, 2008). Arbuscular mycorrhizal fungi can, in fact, colonize haploid gametophytic tissues with different degrees of success: while mosses such as *Physcomitrella patens*, a model organism for basal plants, do not seem to be susceptible to colonization by AMF, many liverworts and hornworts gametophytes are successfully colonized in field (Ligrone *et al.*, 2007) and *in vitro* (Fonseca & Berbara, 2008) conditions, as are some clubmosses and ferns (Boullard, 1979). In ferns, the set-up of an *in vitro* system allowed one of us to demonstrate that only the diploid sporophyte of *Pteris vittata* shows the classical mycorrhizal phenotype (Fig. 1), confirming that the presence of AMF in the fern gametophyte is more erratic (as first noted by Boullard, 1979).

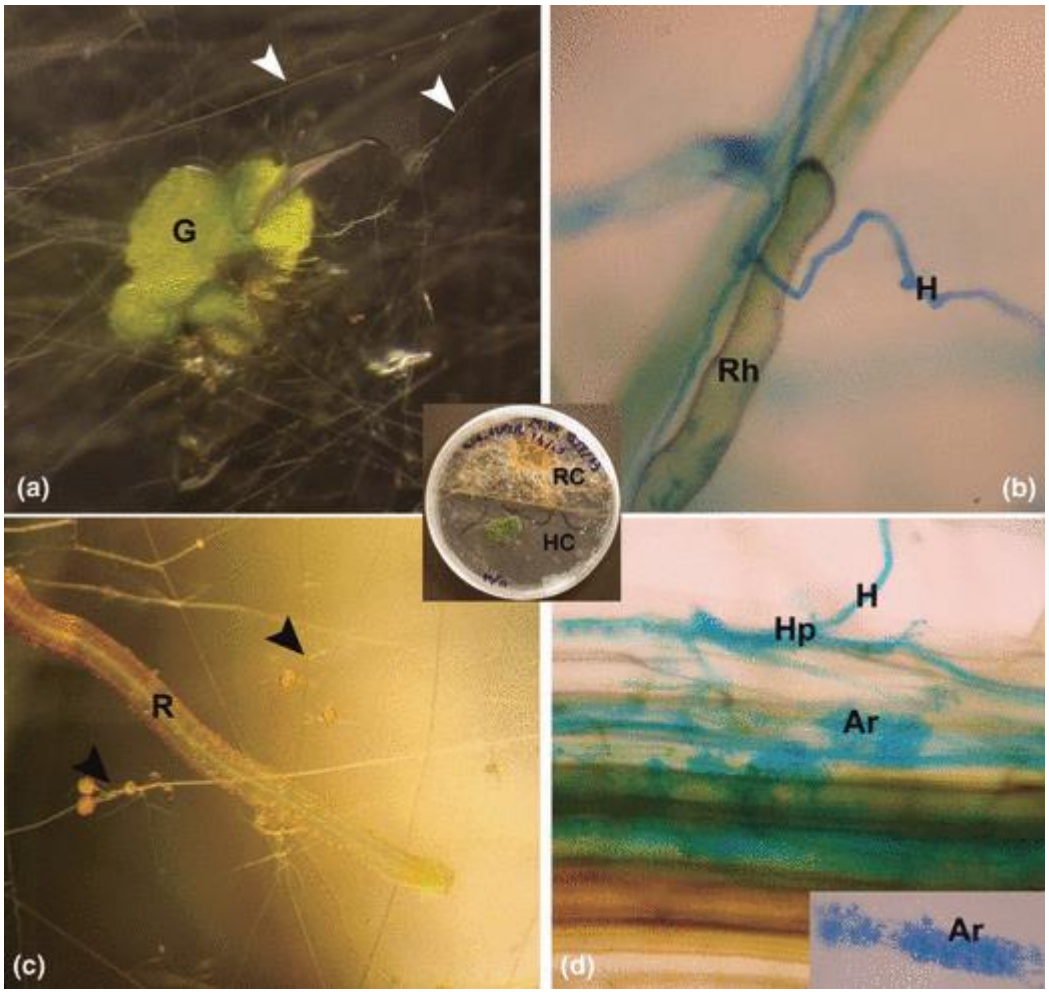

Figure 1. Arbuscular mycorrhizal (AM) associations in the fern *Pteris vittata* are restricted to the diploid sporophyte (M. Novero, *et al.*, unpublished). (a) Gametophytes of *P. vittata*, obtained according to Trotta *et al.* (2007), were transferred to septate Petri dishes, in compartment HC, where *Glomus intraradices* proliferates (arrows) fed by the transformed carrot roots from compartment RC. (b) Hyphae (H) contacted the gametophytic rhizoids (Rh), but colonization events were never observed. (c) Sporophytes (R) developed in the Petri dish after 30 d, while *G. intraradices* produced spores and branched hyphae (arrowheads). (d) The AM hyphae contacted sporophytic root cells, producing hyphopodia (Hp), intracellular hyphae and, lastly, arbuscules (Ar).

The *sym* genes and their evolution

Wang and colleagues provide the first demonstration that the molecular mechanisms of the signalling transduction pathway that control the AM symbiosis are also present in basal land plants, and that AMF colonization is thus a homologous process in all Embryophyta lineages, both in gametophytes and in sporophytes. They investigated the well-known *sym* genes, which have been instrumental in identifying the first steps of symbiosis establishment. Seven of these genes have been identified in *Lotus japonicus* and four in *Medicago truncatula* (Parniske, 2008) and they were demonstrated to be essential in the signalling transduction pathway leading to both AMX and rhizobial symbiosis. It has already been shown that some of these genes were conserved during plant evolution: their presence in monocots, such as rice (*Oryza sativa*), suggests that the associated functions predated the monocots–eudicots divergence (Chen *et al.*, 2009). Starting from this level of knowledge, Wang and colleagues tested the hypothesis of whether orthologs of the *sym* genes were already present in the genome of the basal plants, which spend most of their life cycle in the form of a haploid gametophyte.

They focused on three *sym* genes, which, according to the *Medicago* nomenclature, are described as follows: *DMI1*, coding for a cation channel located in the nuclear envelope; *DMI3*, coding for a calcium/calmodulin-dependent kinase (CCaMK), which acts downstream of the calcium spiking during the perception of the nodulation factor in *Rhizobium* symbiosis; and lastly *IPD3*, coding for a protein that directly interacts with, and is phosphorylated by, CCaMK. Homologs of these genes were successfully isolated from a long list of plants from the major Embryophyta lineages: liverworts, mosses, hornworts, lycophytes, ferns, gymnoperms and, of course, angiosperms. Phylogenetic analyses showed that these genes had been vertically inherited during the evolution of land plants, as the gene phylogenies largely agreed with the Embryophyte phylogeny recently reconstructed from a large body of molecular data (Qiu *et al.*, 2006). To demonstrate the function of such genes, complementation experiments were performed to demonstrate that the *DMI3* genes from liverworts and hornworts could rescue *M. truncatula* dmi3 mutants, which are affected in their mycorrhizal capacities. The transformed *Medicago* roots showed, in fact, some of the expected mycorrhizal features, such as intraradical hyphae, vesicles and sometimes also arbuscules. Moreover, yeast two-hybrid assays also confirmed that the DMI3 proteins from liverworts and hornworts interact with the downstream-acting IPD3 protein in *Medicago*.

These results convincingly suggest that the *sym* genes were already present in ancestral plants and that their function was conserved during evolution. They were maintained during changes in plant life cycles (i.e. the evolution of a gametophyte-dominated or a sporophyte-dominated cycle; Langdale & Harrison, 2008), leading to the concept that root colonization is homologous to that of rootless liverworts and hornworts' thallus. The absence of amplifiable *sym* genes in Charales, green algae that are possible sister taxa to Embryophyta (Becker & Marin, 2009), but the presence in these algae and land plants of a calcium-dependent protein kinase (CDPK; Wang *et al.*) add original information: on the one hand, the discovery points to an ancient innovation that was acquired during terrestrialization; on the other hand, the phylogenetic analysis of *CDPK* and *DMI3* strongly suggests that the latter originated by duplication, with gain of a new function. Freshwater green algal taxa, like the Coleochaetales and the Zygnematales, whose sister position to Embryophyta is still hotly debated (Becker & Marin, 2009), now deserve investigation. Last, the findings of *sym* genes in taxa that do not associate with AMF, such as mosses (*Physcomitrella*) and *Arabidopsis thaliana*, suggest that the corresponding protein may have roles unrelated to the AM symbiosis in some Embryophyta, and this deserves further research.

A window on early land plants?

Of the many questions raised by the work of Wang and colleagues, the first concerns the symbiotic status of the ancestral land plants. Is AM symbiosis a plesiomorphic feature? Indeed, the presence of the *sym* genes in non-AM plants opens the possibility of a repeated recruitment of the same transduction machinery during independent emergence of the AM symbiosis. For example, the repeated emergence of C_4 photosynthesis in angiosperms entailed the parallel evolution of the gene for the key enzyme, the phosphoenolpyruvate carboxylase, to similar, or even identical, amino acid sequences (Christin *et al.*, 2007). Interestingly, in non-AM mosses, except for *Takakia* that associates with AMF, the *sym* genes are undergoing a divergent, positive selection (as also shown by a long branch for non-*Takakia* mosses on Fig. 1 of Wang *et al.*). This pattern suggests that the situation of *sym* genes in non-AM mosses is a derived feature and that the role of *sym* genes in the AM symbiosis is driving their evolution in Embryophyta. Analysis of the sequences and roles of *sym* genes in sister taxa of Embryophyta, if any can be found, would provide additional evidence for this.

Recently, the diversity of morphological features of AM–liverwort associations, and results showing that fungi associated with the extant liverworts mostly belong to Glomerales group A, a relatively recent AM fungal taxon (Russell & Bulman, 2005), pushed Selosse (2005) to consider the possibility of a 'host shifting' in extant bryophytes. Under this hypothesis, independently of the fact that the Embryophyta ancestor was probably mycorrhizal (Selosse & Le Tacon, 1998), several AMF could have recently colonized some basal Embryophyta lineages, so that extant AM symbioses could be secondary reversion or convergence in liverworts and/or hornworts (Selosse, 2005). Further investigations by Ligrone *et al.* (2007), on a larger number of liverworts from different geographic origins, confirmed the dominant presence of Glomerales from group A, although a Diversisporales-related sequence was also detected. These data again suggested that extant liverworts are more frequently associated with recent AM fungal taxa, in line with the previous hypothesis. However, unpublished results by M. Naumann, A. Desirò and P. Bonfante on *Conocephalum*, a common liverwort species, identified sequences related to diverse and more ancient taxa, such as Diversisporales and Archeosporales. Interestingly, these results were obtained by using long primers for the small subunit (SSU) region amplification recently set up by Lee *et al.* (2008), perhaps overcoming the technical limitations of previous primer sets.

In conclusion, the phylogenetic analysis by Wang and colleagues, demonstrating the presence of the *sym* genes in basal plants and the presence in liverworts of ancient VA fungal taxa, strongly support the concept that AM symbioses are likely to be homologous, that is, a trait derived from the common ancestor of all Embryophyta (Selosse & Le Tacon, 1998; Kottke & Nebel, 2005; Wang & Qiu, 2006). Thus, (1) AM symbiosis should now join the synapomorphies (i.e. traits shared by two or more taxa and their last common ancestor) of Embryophyta in textbooks and (2) as a result, extant non-mycorrhizal lineages did secondarily (and independently) lose AM symbiosis during Embryophyta evolution. Ironically, the mycorrhizal association is more ancient than the roots defining it (-rhiza), which arose only within vascular plants. However, this does not necessarily entail that the morphology of the plants' common ancestor and its AM association were close to that of extant liverworts and hornworts (Selosse, 2005; Bonfante & Genre, 2008). In this respect, one may hope to unravel well-preserved fossils and rich *Lagerstätten*, older than the 400-million-yr-old Rhynie Chert where Embryophyta and AM symbioses had already diversified (Boullard & Lemoigne, 1971; Krings *et al.*, 2007).

Acknowledgements

We thank M. Novero, A. Trotta, M. Naumann and A. Desirò for sharing unpublished data, and Professor Yin-Long Qiu for critical reading of this commentary.

References

- Becker B, Marin B. 2009. Streptophyte algae and the origin of embryophytes. Annals of Botany 103: 999–1004.
- Bonfante P, Genre A. 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends in Plant Science 13: 492–498.
- Boullard B. 1979. Considérations sur la symbiose fongique chez les Ptéridophytes. Syllogeus 19: 1–58.
- Boullard B, Lemoigne Y. 1971. Les champignons endophytes du "Rhynia gwynne-vaughanii" K. et L. Étude morphologique et déductions sur leur biologie. Botaniste 54: 49–89.
- Bucher M, Wegmüller S, Drissner D. 2009. Chasing the structures of small molecules in arbuscular mycorrhizal signaling. Current Opinion in Plant Biology 12: 500–507.
- Chen C, Fan C, Gao M, Zhu H. 2009. Antiquity and function of CASTOR and POLLUX, the twin ion channel-encoding genes key to the evolution of root symbioses in plants. Plant Physiology 149: 306–317.
- Christin PA, Salamin N, Savolainen V, Duvall MR, Besnard G. 2007. C4 Photosynthesis evolved in grasses via parallel adaptive genetic changes. Current Biology 17: 1241–1247.
- Fonseca HMAC, Berbara RLL. 2008. Does Lunularia cruciata form symbiotic relationships with either Glomus proliferum or G. intraradices? Mycological Research 112: 1063–1068.
- Kidston R, Lang WH. 1921. On Old Red Sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part V. The Thallophyta occuring in the peat-bed; the succession of the plants throughout a vertical section of the bed, and the conditions of accumulation and preservation of the deposit. Transactions Royal Society of Edinburgh 52: 855–902.
- Kottke I, Nebel M. 2005. The evolution of mycorrhiza-like associations in liverworts: an update. New Phytologist 167: 330–334.
- Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ. 2007. An alternative mode of early land plant colonization by putative endomycorrhizal fungi. Plant Signalling and Behaviour 2: 125–126.
- Langdale JA, Harrison CJ. 2008. Developmental transitions during the evolution of plant form. In: MinelliA, FuscoG, eds. Evolving pathways: key themes in evolutionary developmental biology. Cambridge, UK: Cambridge University Press, 299–316.
- Leake JR, Cameron DD, Beerling DJ. 2008. Fungal fidelity in the mycoheterotroph-toautotroph life cycle of Lycopodiaceae: a case of parental nurture? New Phytologist 177: 572–576.
- Lee J, Lee S, Young JPW. 2008. Improved PCR primers for the detectionand identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65: 339–349.
- Ligrone R, Carafa A, Lumini E, Bianciotto V, Bonfante P, Duckett JG. 2007. Glomeromycotean associations in liverworts: a molecular, cellular and taxonomic analysis. American Journal of Botany 94: 1756–1777.
- Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Reviews Microbiology 6: 763–775.
- Qiu Y-L, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J et al. 2006. The deepest divergences in land plants inferred from phylogenomic evidence. Proceedings of the National Academy of Sciences, USA 103: 15511–15516.
- Russell J, Bulman S. 2005. The liverwort Marchantia foliacea forms a specialized symbiosis with arbuscular mycorrhizal fungi in the genus Glomus. New Phytologist 165: 567–579.
- Selosse M-A. 2002. Prototaxites: A 400 Myr old giant fossil, a saprophytic holobasidiomycete, or a lichen? Mycological Research 106: 642–644.
- Selosse M-A. 2005. Are liverworts imitating mycorrhizas? New Phytologist 165: 345–349.

- Selosse M-A, Le Tacon F. 1998. The land flora: a phototroph–fungus partnership? Tree 13: 15–20.
- Simon L, Bousquet J, Levesque RC, Lalonde M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363: 67–69.
- Taylor WA, Free C, Boyce C, Helgemo R, Ochoada J. 2004. SEM analysis of Spongiophyton interpreted as a fossil lichen. International Journal of Plant Sciences 165: 875–881.
- Trotta A, Mantovani M, Fusconi A, Gallo C. 2007. In vitro culture of Pteris vittata, an arsenic hyperaccumulating fern, for screening and propagating strains useful for phytoremediation. Caryologia 60: 160–164.
- Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299–363.
- Wang B, Yeun LH, Xue J-Y, Liu Y, Ané J-M, Qiu Y-L. 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytologist 186: 514–525.
- Wellman CH, Osterloff PL, Mohiuddin U. 2003. Fragments of the earliest land plants. Nature 425: 282–285.
- Winther JL, Friedman WE. 2008. Arbuscular mycorrhizal associations in Lycopodiaceae. New Phytologist 177: 790–801.